

1. Five (5) specific probes are synthesized to specifically bind to specific regions of mRNA in different bacterial species; Eub 338, Alflb, Bet 42a and Gam 42a, HGC, and CF. The probes also contain a fluorescent marker.

2. "Cells from activated sludge samples or pure cultures were mechanically disrupted with glass beads in combination with sonification". In this process the cell membrane is ruptured, and most, if not all, of the cell contents are freely accessible in solution. Col. 1, bottom of page 1717.

3. The freed mRNA are then "purified by phenol extraction and then separated by ethanol precipitation. The isolated mRNA is then immobilized (fixed) on nylon membranes.

4. The specific probes synthesized in step 1 are mixed with divided samples of the fixed mRNA, to form a probe-target complex. The amount of probe-target complex is then quantified by fluorescence microscopy. A photo-image was generated and image analysis software was used to quantify the probe-target complex. Col. 1, middle of page 1717.

There is no teaching in Manz, that the specific probes can come in contact and recognize a mRNA target in "whole cells" as claimed, that is, without the need for cell lysis as described in step 2 above. Also, there is no teaching in Manz that once the specific probe locates and binds the target mRNA to form a hybridized specific probe, the probe is then extracted from the target by "adding a denaturing agent to denature the probe-target complex, as claimed. Instead, the probe-target complex remains intact during quantification by fluorescence microscopy.

With respect to De Los Reyes, the Advisory Action states that De Los Reyes "teaches adding different concentration of formaldehyde, which is a denaturing agent, to optimize FISH conditions including hybridization and such conditions", citing p. 1113, col. 2, first paragraph. However, the different concentrations of formaldehyde were used to fix the cells, not to denature the probe-target complex, as claimed.

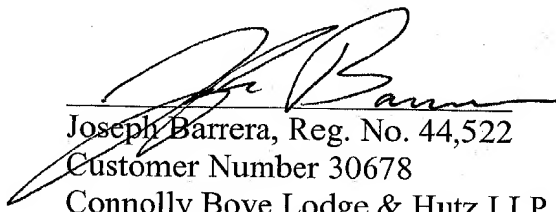
Like Manz, quantification of targets occurs with the probe-target intact. Again, the amount of binding probe is quantified by fluorescence microscopy. As a result, there

is no teaching in De Los Reyes, to extract the "hybridized probes from their target by adding a denaturing agent", as claimed, followed by their quantitative measurement of the probe, absent any target.

Accordingly, Applicant requests that this rejection be withdrawn.

The Director is hereby authorized to charge any fees, or credit any overpayment, associated with this communication, including any extension fees, to CBLH Deposit Account No. 22-0185.

Respectfully submitted,



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Version with markings to show changes made.

In the Claims:

Claims 1, 5-7 and 14 were amended as follows:

1. (Twice Amended) A method of qualitative and quantitative analysis of microbial population(s) comprising:
contacting microorganisms present in a sample with at least one specific probe to form a probe-target complex, wherein the specific probe recognizes a RNA target sequence under conditions favorable to *in situ* hybridization in whole cells,
extracting the hybridized specific probes [that are hybridized by separation] from their target by adding a denaturing agent to denature the probe-target complex, and
detecting the extracted probes and measuring the amount thereof or their respective amounts.
5. (Thrice-amended) [Method] A method according to Claim 3 wherein said specific or said universal probe is a *mRNA*-targeted probe.
6. (Thrice-amended) A method according to Claim 1, [wherein] further comprising extracting said microorganisms in said sample [are extracted from said sample] by centrifugation.
7. (Twice-amended) A method according to Claim 1, wherein said contacting is performed [followed by] following fixation of said whole cells.
14. (Thrice-amended) A method according to Claim 1, wherein extracting of the hybridized probes includes [adding a denaturing agent to denature the probe-target complex, and] extracting at a temperature higher than the melting temperature of the specific probe under consideration.